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Claims

1. A probe molecule comprising single stranded or partially double stranded nucleic acid, wherein said probe comprises: a target complementary portion, a template portion, at least one enzyme acting portion, with or without a 3' end block portion and wherein said template portion comprises two identical or nearly identical sequences, which are separated by at least one enzyme acting portion when said probe is linear.
2. A probe according to claim 1, wherein said single stranded or partially double stranded nucleic acid is a linear molecule.
3. A probe according to claim 1, wherein said single stranded or partially double stranded nucleic acid is a circular molecule.
4. A probe according to claim 3, wherein said probe is circular probe, wherein said circular probe comprises one template portion.
5. A probe according to claim 1, wherein said enzyme acting portions comprise a RNA polymerase promoter.
6. A probe according to claim 1, wherein said enzyme acting portions comprise RNase H acting sequences.
7. A probe according to claim 1, wherein said enzyme acting portions comprise a nuclease digestion site, wherein said nuclease digestion site support digesting opposite strand of said probe when double stranded.
8. A probe according to claim 1, wherein said at least one enzyme acting portion comprises a restriction enzyme site.

9. A probe according to claim 7, wherein said enzyme acting portions comprise the combination of the RNase H acting sequences and the RNA polymerase promoter or the combination of the RNase H acting sequences and said nuclease digestion sites or the combination of said nuclease digestion sites and the RNA polymerase promoter or the combination of more than one of said nuclease digestion sites.
10. A probe according to claim 7, wherein said nuclease digestion site comprises modified nucleotides, whereby said digestion site on the probe is resistant to nuclease cleavage and the opposite unmodified strand is sensitive to cleavage.
11. A probe according to claim 10, wherein said modified nucleotides comprise phosphorothioate linkages.
12. A probe according to claim 7, wherein said nuclease digestion sites comprise restriction site having a restriction enzyme recognition sequence and a cleavage site.
13. A probe according to claim 12, wherein said restriction site comprises a type IIS restriction enzyme site.
14. A probe according to claim 13, wherein the enzyme cleavage site of said type IIS restriction site is located on target complementary portion.
15. A probe according to claim 14, wherein said type IIS restriction enzyme cleavage site corresponds to a SNP site, mutation nucleotide, methylation nucleotide or splicing site.
16. A probe according to claim 13, wherein said type IIS restriction site is the Fok I site.
17. A probe according to claim 1, comprising helper primer(s), wherein said helper primer comprises at least one portion complementary or substantially complementary to a part of said probe.

ART 24 AMDT

18. A probe according to claim 17, wherein said helper primer comprises a 3' end blocking moiety, whereby the 3' end of said helper primer is not extendible by a DNA polymerase.
19. A probe according to claim 17, wherein said helper primer does not comprise a 3' end blocking moiety, whereby the 3' end of said helper primer is extendible by a DNA polymerase.
20. A probe according to claim 17, wherein said helper primer comprises sequence complementary to the enzyme acting portion(s) with or without flanking sequences or to part of the enzyme acting portion(s) of said probe, whereby hybridization between said helper primer and said probe makes the enzyme acting portion(s) double stranded or partially double stranded.
21. A probe according to claim 17, wherein said helper primer comprises 3' end sequence complementary to a sequence 3' to one of the enzyme acting portions of said probe.
22. A probe according to claim 17, wherein said helper primer further comprises target complementary portion(s), wherein the target region(s) complementary to said helper primer is adjacent or substantially adjacent to the target region complementary to said probe.
23. A probe according to claim 22, wherein said helper primer comprises 3' and 5' target complementary portions, wherein the target region complementary to said probe is located in the middle of the target regions complementary to said helper primer and is adjacent or substantially adjacent to the target regions complementary to said helper primer.
24. A probe according to claim 1, wherein said target complementary portion comprises sequence complementary or substantially complementary to a target region of interest, whereby said target complementary portion of said probe hybridizes to said

target region of interest and becomes double stranded, whereby one or more than one or part of the enzyme acting portion(s) of said probe is partially or fully functional.

25. A probe according to claim 1, wherein said enzyme acting portion(s), said target complementary portion and said template portion(s) of said probe overlap each other or have one portion embedded in other portions.

26. A probe according to claim 1, wherein said target complementary portion and/or said enzyme acting portion(s) and/or said template portion(s) of said probe comprise modified nucleotides, whereby modified nucleotides are resistant to nuclease cleavage.

27. A probe according to claim 1, wherein said target complementary portion and/or said enzyme acting portion(s) and/or said template portion(s) of said probe comprise chimeric RNA and DNA.

28. A probe according to claim 1, wherein said probe comprises a catalytically inactive antisense sequence complementary to a DNA enzyme in any place of the circular probe or within the 5' template portion with or without surrounding portion sequences of the linear probe.

29. A probe according to claim 28, wherein said DNA enzyme is 10-23 DNAzyme.

30. A probe according to claim 28, wherein said DNA enzyme is 8-17 DNAzyme.

31. A probe according to claim 1, wherein said 3' end block portion is chemical moiety, whereby 3' end of the probe is not extendible by a DNA polymerase.

32. A probe according to claim 1, wherein any end of said probe and/or helper primer is attached on a solid support.

ART 34 AMDT

33. A method of detecting a target nucleic acid sequence or multiple target nucleic acid sequences of interest in a sample, the method comprising the steps of:

- (a) contacting probes or a set of probes in accordance with any one of the preceding claims with a nucleic acid sample under suitable hybridization conditions, wherein the target complementary portions of said probes or the target complementary portions of both said probes and helper primers (if present) hybridize the target sequence(s) and become double stranded, whereby one or more than one or part of the enzyme acting portion(s) of said probe is partially or fully functional;
- (b) causing all enzyme acting portions of said probes double stranded and fully functional;
- (c) treating said probes containing double stranded enzyme acting portion(s) so as to produce the single stranded end product (SSEP);
- (d) annealing said SSEP to free probes and causing all enzyme acting portions of said probes double stranded and fully functional, wherein said free probes are the same probes used in step (a);
- (e) repeating steps (c) and (d), whereby said probes are converted to double stranded or partially double stranded form, and multiple copies of said SSEP are produced repeatedly; and
- (f) detecting directly or indirectly the end products so produced: double stranded end product, SSEP and pyrophosphate (PPi).

34. A method according to claim 33, wherein said method is performed in a single reaction or in separated reactions.

35. A method according to claim 33, wherein said target nucleic acid is RNA and said step (a) causes one of the enzyme acting portion, the RNase H digesting sites, double stranded and functional; wherein said step (b) comprises: digesting RNA strand by RNase H, extending the 3' end of partially digested strand using said probe as template by a DNA polymerase, whereby all other enzyme acting portions on said probes become double stranded and functional.

36. A method according to claim 35, wherein said extending the 3' end of partially digested strand further comprises strand displacing by said DNA polymerase or other strand displacement factors.

37. A method according to claim 35, wherein said other enzyme acting portions on said probes comprise restriction site or RNA polymerase promoter or both restriction site and RNA polymerase promoter.

38. A method according to claim 33, wherein one of said enzyme acting portions is restriction site and is located on the target complementary portion of said probe, said step (a) causes said restriction site double stranded and fully functional, wherein said step (b) comprises: digesting opposite strand of said probes on said restriction site by a restriction enzyme, and extending the 3' end of the digested strand using said probe as template by a DNA polymerase, whereby all other enzyme acting portions on said probes become double stranded and functional.

39. A method according to claim 38, wherein said extending the 3' end of the digested strand further comprises strand displacing by said DNA polymerase or other strand displacement factors.

40. A method according to claim 38, wherein said other enzyme acting portions on said probes comprise restriction site or RNA polymerase promoter or both restriction site and RNA polymerase promoter.

41. A method according to claim 38, wherein said restriction site is the only enzyme acting portion on said probe.

42. A method according to claim 33, wherein one of said enzyme acting portions is type IIS restriction site, wherein the cleavage site of said type IIS restriction site is located on target complementary portion of said probe and the recognition site of said type IIS restriction site is on either side of target complementary portion of said probe;

wherein step (a) causes the target complementary portions of said probe double stranded, whereby a functional cleavage site of said type IIS restriction site is formed; wherein said step (b) comprises: annealing helper primers to said probes and causing said recognition sequence of said type IIS restriction site double stranded.

43. A method according to claim 42, wherein said annealing helper primers to said probes and causing said recognition sequence of said type IIS restriction site double stranded comprises: annealing said helper primers directly to said type IIS restriction enzyme recognition sequence with or without flanking sequences whereby double stranded recognition sequence of said type IIS restriction site is formed.

44. A method according to claim 42, wherein said annealing helper primers to said probes and causing said recognition sequence of said type IIS restriction site double stranded comprises: annealing the 3' end sequence of said helper primer to a sequence 3' to said type IIS restriction recognition sequence and extending the 3' end sequence of said helper primer by a DNA polymerase using said probe as template, whereby double stranded recognition sequence of said type IIS restriction site is formed.

45. A method according to claim 33, wherein in said step (a) the target complementary portions of said probes hybridize to free 3' end(s) of the target sequence(s), said step (b) comprises: extending said free 3' end(s) of the target sequence(s) by a DNA polymerase using said probes as templates, whereby other enzyme acting portions on said probes become double stranded and functional.

46. A method according to claim 33, wherein said enzyme acting portions of said probe comprise a restriction site, said step (c) comprises: digesting opposite strands of said probes on said restriction site by a restriction enzyme, extending the 3' end of the digested strand by a DNA polymerase, and repeating said digesting and said extending, whereby multiple copies of SSEP DNA are produced.

ART 34 AMDT

47. A method according to claim 46, wherein said extending the 3' end of the digested strand further comprises strand displacing by said DNA polymerase or other strand displacement factors.

48. A method according to claim 33, wherein said enzyme acting portions of said probe comprise RNA polymerase promoter, said step (c) comprises: repeated transcription by the RNA polymerase acting on said RNA polymerase promoter, whereby multiple copies of SSEP RNA are produced.

49. A method according to claim 33, wherein said enzyme acting portions of said probe comprise both restriction site and RNA polymerase promoter, said step (c) comprises: digesting opposite strands of said probes on said restriction site by a restriction enzyme, extending the 3' end of digested strands by a DNA polymerase, repeating said digesting and said extending, whereby multiple copies of SSEP DNA are produced, and repeated transcription by the RNA polymerase acting on said RNA polymerase promoter, whereby multiple copies of SSEP RNA are produced.

50. A method according to claim 49, wherein said extending the 3' end of the digested strand further comprises strand displacing by said DNA polymerase or other strand displacement factors.

51. A method according to claim 33, wherein said SSEP are DNA molecules or RNA molecules or both DNA and RNA molecules, said step (d) comprises: annealing said SSEP to sequence portions of free probes and extending the 3' ends of said SSEP using said free probes as templates, whereby all enzyme acting portions of said probes become double stranded and functional.

52. A method according to claim 33, wherein said SSEP are RNA molecules, said step (d) comprises: annealing said SSEP to sequence portions of free probes, digesting said SSEP by RNase H, and extending the 3' end of partially digested SSEP using said free probes as templates, whereby all enzyme acting portions become double stranded and functional.



ART 34 AMEND

- 55 -

53. A method according to claim 33, wherein said probes are circular molecules, the sequences of said SSEP comprise one or more than one sequence unit that is complementary to said probes, step (d) comprises: annealing said SSEP to the whole or parts of said free probes, whereby said enzyme acting portions become double stranded and functional.

54. A method according to claim 33, wherein said template portions comprise antisense DNA enzyme, said method produces multiple copies of single stranded functional sense DNA enzyme, said step (f) of detecting single stranded end product comprises: including a RNA or DNA-RNA chimeric reporter substrate in the reaction, wherein said RNA or DNA-RNA chimeric reporter substrate comprises fluorescence resonance energy transfer fluorophores incorporated on either side of a DNAzyme cleavage site, cleaving said reporter substrate by sense DNA enzyme, whereby cleavage of said reporter substrate produces an increase in fluorescence signal.

55. A kit for use in detecting a target nucleic acid sequence or multiple target nucleic acid sequences of interest in a sample, said kit comprising: said a set or sets of probes as defined in any one of claims 1 to 32, said helper primers, said detection substrate, said restriction enzymes, said RNA polymerase, said RNase H, said DNA polymerase, buffers, dNTPs, NTPs.